



PCR Technology

U.S. EPA | SCIENCE AT THE EPA NEW ENGLAND REGIONAL LABORATORY

SCIENCE lies at the heart of the mission of the U.S. Environmental Protection Agency (EPA). The Agency must rely on cutting edge research, accurate measurements and effective technology to implement its programs to protect the environment and human health. Without sound science and credible data, EPA can not wisely set environmental and health standards, clean up contaminated sites, measure ambient air and water quality conditions, or identify the new technologies or practices that will reduce releases to the environment. These fact sheets share with you some of our EPA New England's laboratory capabilities and exemplify some of the very best science we do to meet our agency mission.

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GOAL:

EPA's New England Regional Laboratory, in collaboration with EPA's Office of Research and Development and Office of Water, is developing innovative cost-effective analytical capabilities to enhance existing microbial assessment of water resources by the application of a biochemical technology for rapid, same-day detection and quantification of naturally occurring microbes and microbial pollution.

PROGRESS:

PCR (Polymerase Chain Reaction) is a biochemical genetic technique that mimics the natural cellular process of nucleic acid duplication in living cells (either DNA: deoxyribonucleic acid, or RNA: ribonucleic acid). DNA and RNA are made up of genes comprised of unique sequences of nucleotide bases which can be detected by PCR analysis, thus identifying a specific strain of bacteria or a specific animal, like birds, cows, or humans.

The regional laboratory's quantitative Real-Time Polymerase Chain Reaction (qPCR) technology capability is an innovative and developing scientific tool that has significant potential for assisting EPA scientists in determining regulatory responsibility for fecal coliform pollution in the region's water resources. EPA New England is collaborating with EPA's Office of Research and Development and Office of Water to evaluate the best protocols and test methods for utilizing qPCR in support of EPA's mission. To date, EPA New England has established three different real-time qPCR assays capable of analyzing fecal indicators in marine and fresh water samples and is focusing on microbial source tracking of fecal contaminants in marine waters off urban beaches in metropolitan Boston, Massachusetts.

Almost one quarter of the 2,840 waterbodies in New England listed as "impaired" are so classified because they do not meet water quality criteria for bacteria. Traditional microbial test methods limit state, federal and tribal regulators' ability to implement appropriate control measures and/or to assess human health risks. Traditional microbial methods have significant holding-time constraints as well as delayed results due

to a 24 hour minimum time for analysis completion. These methods also cannot distinguish specific differences between the sources of microbial pollution. The advantage of qPCR is that, in most cases, analysis can be completed in four hours or less, and scientists do not have the same 6 hour holding-time limitation required by culture-based test methods. In addition, qPCR can identify host-specific pollution indicators, or those bacteria that are only associated with specific species.

The laboratory is also developing PCR techniques to detect and quantify naturally occurring beneficial bacteria that digest chemical pollutants and change them into harmless gases. These methods will support screening assessments of groundwater microbial communities capable of bioremediation, or the natural degradation of industrial solvents to non-hazardous break-down products. This will be a valuable tool to support the cleanup of hazardous waste sites.

BENEFITS:

Scientists at the EPA's New England Regional Laboratory are continuing in the development and validation of routine and repeatable analytical qPCR methods. These methods will allow EPA scientists to rapidly determine whether or not samples contain DNA from known targets, whether those targets are fecal pollution indicators, like E. coli, or bacteria capable of metabolizing hazardous waste, like the anaerobe Dehalococcoides ethenogenes. By helping identify sources of microbial contamination, qPCR will allow regulators to identify more effective measures to mitigate pollution.

